FISH EMBRYOS AS ALTERNATIVE MODELS IN TOXICOLOGY: A REVIEW

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EMBRIONI RIBA KAO ALTERNATIVNI MODEL U TOKSIKOLOGIJI: REVIJALNI PREGLED

Apstrakt

Povećan broj ksenobiotika ugrožava životnu sredinu. Ova bioaktivna jedinjenja dospevaju u vodenu sredinu na različite načine i imaju značajan uticaj na akvatični živi svet. Za procenu opasnosti i rizika industrijskih hemikalija, proizvoda zaštite bilja, biocida, dodataka u hrani i lekova koriste se testovi na životinjama. Akutni test toksičnosti za ribe je obavezni korak u procesu ispitivanja toksičnosti hemikalija. Međutim njihova ekotoksikološka relevantnost je pod znakom pitanja. Povrh toga ribe se u ovim testovima podvrgavaju značajnom bolu i patnji. U skladu sa filozofijom 3r (redukcija/smanjenje, refinement/prečišćavanje, replacement/zamena) embrioni riba se smatraju metodom zamene ili prečišćavanja budući da ovi razvojni stadijumi nisu zakonski zaštićeni (Direktiva 2010/63/EU) i izgleda da osećaju manju bol i patnju.

Cilj ovog pregleda bio je da se sumiraju dostupni podaci o testovima toksičnosti na embrionima riba i ukaže na moguće perspektive korišćenja ovog modela u savremenoj toksikologiji. Princip testa toksičnosti sa embrionima riba zasniva se na izloženosti vijabilnih embriona ksenobioticima, od oplođenja do kraja embriogeneze. Test toksičnosti sa embrionima riba validiran je u OECD studiji i prihvaćen kao OECD TG 236 96-h test za procenu toksičnosti za embrione riba. Određuje se letalnost koja je označena koagulacijom embriona, nedostatkom somita, ispravljanja repa ili otkucaja srca. Povrh smrtnosti, složeniji pokazatelji kao što je razvoj očiju, prisustvo krvotoka ili razvojne malformacije mogu se uočiti na stadijumima izvaljenih eleutero embriona. Mogućnost praćenja različitih pokazatelja čini embrione riba odličnim modelom za ispitivanje i razumevanje mehanizama toksičnosti i indikatorom mogućih nepovoljnih i dugotrajnih efekata.

Na osnovu izvedenih istraživanja, test toksičnosti sa embrionima riba je razumna alternativa akutnom testu toksičnosti na ribama. Raspon mogućih aplikacija je značajan i

ima mnogo perspektiva u budućnosti: kao što je dalja identifikacija molekularnih markera – indikatora načina delovanja ili uspostavljanje veze među efektima u kratkotrajnim testovima sa embrionima i dugotrajnih efekata na individue. Test toksičnosti sa embrionima riba takođe može da doprinese smanjenju broja eksperimenata na životinjama što je jedan od osnovnih principa EU legislative u oblasti dobrobiti.

Ključne reči: testovi toksičnosti sa životinjama, REACH, Danio rerio. Test akutne toksičnosti na ribama, vodena sredina
Keywords: Animal toxicity test, REACH, Danio rerio, fish acute toxicity test, aquatic environment

INTRODUCTION

In EU, most industrial chemicals are regulated by the Regulation (EC) No 1907/2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals, known as REACH (EU, 2006). Animal tests are used to assess the environmental hazard identification and risk assessment of industrial chemicals, plant protection products, biocides, feed additives and pharmaceuticals (Scholz et al., 2013). Bioactive compounds can reach the aquatic environment in many ways and have considerable effects on aquatic biota. Fish play a critical role in aquatic food webs and are an important food source for humans. Fish have been accepted as vertebrate representatives for the aquatic environment in acute as well as chronic toxicity tests and are also used to monitor the quality of effluents and surface waters (Lammer et al., 2009). The fish acute toxicity test (FAT) is a mandatory step in environmental hazard and risk assessment of chemicals. However, its ecotoxicological relevance is questionable. Value of LC_{50} may vary among different species and short-term exposure to high concentrations of toxicant in nature is not expected. The only exception may represent accidental spills. Moreover, fish are subjected to considerable pain and suffering (Nagel, 2002). Growing number of chemicals consumption brings request for novel and reliable methods identifying possible hazard of chemical compounds (Oxendine et al, 2006).

The aim of this review was to summarize available data on fish embryo toxicity tests (FET) and introduce their possible perspectives in modern toxicology.

MATERIALS AND METHODS

The review summarizes available data on fish embryo toxicity tests and introduce their possible perspectives in modern toxicology.

Available research articles dealing with fish embryo toxicity tests were collected and studied. The research findings were summarized and the review was completed.

RESULTS AND DISCUSSION

In accordance with reduction, refinement and replacement philosophy (the 3Rs; Russell and Burch, 1959) fish embryos are considered as replacement or refinement method since these developmental stages are not legislatively protected (Directive 2010/63/EU) and are

likely to experience less pain or suffering. (EFSA, 2005). The idea of embryo assay has been suggested by Schulte and Nagel (1994). The principle insists in the exposition of viable embryos, from fertilization until the completion of embryogenesis (48 h), to xenobiotics when various responses are recorded. Coagulation of eggs and embryos, lack of somite formation, non-detachment of the tail and lack of heartbeat are assessed to be lethal and are observed in order to determine LC_{50} (Nagel, 2002). Additionaly, sub-lethal endpoints such as completion of gastrula, formation of somites, development of eyes, spontaneous movement, hearbeat/ blood circulation, pigmentation and oedema may be recorded to indicate the mode of action of toxic coumpound. The analysis can also include the screening for developmental disorders to indicate teratogenic effects (Nagel, 2002): malformations of the head, otoliths, tail and heart, modified structure of the corda, scoliosis, rachischisis, deformity of yolk, growth-retardation and length of tail. Although the FET was originally designed as an alternative to the FAT, the FET was internationaly standardized. In Germany, previous acute fish toxicity testing of effluents was replaced by a standardized 48-h wastewater test with zebrafish (*Danio rerio*) embryos (DIN 2001, ISO 2007) in 2005. The FET was also validated during an OECD validation study and adopted as OECD TG 236 as a 96-h test to assess toxicity using zebrafish embryonic stages (Braunbeck et al., 2014). In some studies the extended version of 96-h FET has been prioritized to cover the critic phase of hatching and eleutheroembryo (the free embryo) development (e.g. Carlsson et al., 2013 or Selderslaghs et al., 2009). In accordance with Directive 2010/63/EU, the earliest life stages of fish are not legislatively protected until they feed independently. The way of feeding (endogenous or exogenous) also distinguishes the developmental phases of embryo (egg), eleutheroembryo (feeding off the yolk sac) and larvae (exogenous feeding) (Belanger et al., 2010). There is a strong need to distinguish between non-protected and protected life intervals among different fish species. According to Strähle et al. (2012) zebrafish are capable of independent feeding at 120 h post-fertilization. Belanger et al. (2010) suggest that tests conducted on zebrafish embryo should be terminated between 24 and 48 h after hatching. However, at 144 hpf active food uptake was not documented in all individuals (Belanger et al., 2010) and the non-feeding eleutheroembryo stage can be interpreted as an extension phase of embryonic development (Strähle et al., 2012; Brauenbeck et al., 2014).

Zebrafish (*Danio rerio*) belongs among prominently used toxicological models . Main benefits of zebrafish usage are small size and optimum breeding and maintenance conditions. The zebrafish mature rapidly (within 3 month at 26 °C) and are capable of laying 50–200 eggs every day. The embryos are high in fecundity and their transparency as well as well described development (e.g. by Kimmel el al., 1995) are big advantages of using zebrafish embryos in fish embryo toxicity tests (Hill et al., 2005; Nagel, 1993). Even though the zebrafish is the most used species in this context, additional species such as fathead minnow (*Pimephales promelas*) and medaka (*Oryzias latipes*) should be pursued in the future (Belanger et al., 2013).

In accordance with study comparing results from FAT and FET conducted by Nagel (2002) the fish embryo test is a reasonable alternative to fish acute toxicity test. Recently, Lammer et al. (2009) and Belanger et al. (2013) summarized the FET and FAT studies in order to understand their potentional relationships and the FET applicability in chemical testing. Their results were in accordance with those from Nagel (2002) and provided scientific support for the FET as an alternative to fish acute toxicity test.

CONCLUSIONS

The fish embryo toxicity test is a reasonable alternative to fish acute toxicity test with wide range of future perspectives. The fish embryo toxicity tests could also contribute to the reduction of animal experiments, which is one of the core principles of EU welfare legislation.

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